Reference: *Biol. Bull.* **164:** 279–298. (April, 1983)

SPECIFIC IDENTIFICATION AND ASSESSMENT OF DISTRIBUTION AND ABUNDANCE OF EARLY PENAEID SHRIMP LARVAE IN THE GULF OF CARPENTARIA, AUSTRALIA

PETER C. ROTHLISBERG, CHRISTOPHER J. JACKSON, AND ROBERT C. PENDREY

Division of Fisheries Research, CSIRO Marine Laboratories, P. O. Box 120, Cleveland, Queensland 4163, Australia

Abstract

Methods for obtaining eggs and rearing larvae of penaeid shrimp at sea, in remote areas, were developed and used to build a comprehensive larval reference collection for taxonomic purposes. Because of the large amount of morphological variation within species and character overlap between species, a multivariate numerical identification technique, discriminant analysis, was tested using larvae of the four species of *Penaeus: p. esculentus; P. latisulcatus; P. merguiensis;* and *P. semisulcatus,* in our reference collection. The overall accuracy of the technique is high (>85%) and can be increased by narrowing the range of natural morphological variation considered, at the expense of decreasing the number of larvae positively identified. Application of the technique to the first zoeal larvae in our plankton collections from the Gulf of Carpentaria, Australia, shows discrete, discontinuous larval distributions which delimit the spawning activity of the four species to a degree not possible by sampling and histological examination of the adult shrimp.

Introduction

The family Penaeidae makes up approximately 70% of the world's prawn catch (estimated from Table II in Wickins, 1976). These penaeid stocks are also characterized by very large fluctuations in size (Kirkegaard, 1975), with little apparent relationship between spawner abundance and recruitment strength (Neal, 1975; Rothschild and Gulland, 1982). This is not surprising considering that most commercially important species are fecund, short-lived and have a complex life cycle which involves a variety of distinct habitats (Kutkuhn, 1966; Garcia and Le Reste, 1981). After the demersal eggs hatch the pelagic larvae go through a series of larval stages which are lecithotrophic, herbivorous and carnivorous before reaching their nearshore or estuarine nursery areas and settling out of the water column. Because of the high fecundity and complex larval life history, it is probable that a large amount of the year-to-year variability in stock size is accounted for by factors affecting larval and postlarval mortality. Detailed studies of the larval ecology, as well as precise assessment of reproductive activity based on distribution and abundance of early larval stages, have been hampered by the inability to identify the early larval stages found in the plankton (Racek, 1959; Temple and Fischer, 1967; Kutkuhn *et al.,* 1969; Subrahmanyam, 1971; Sandifer and Eldridge, 1976).

Methods for identifying the genera of penaeid zoea larvae are well established. The characters which have been found to be most useful relate to the setal distri-

Received 16 August 1982; accepted 5 January 1983.
CSIRO Marine Laboratories Reprint No. 1333.
All measurements are presented in ocular divisions (O.D.) where 1.0 ocular division = 0.118 mm.

bution on the endopod of the second antenna (Cook, 1966a; Hassan, 1974). To date however, there has been no success in identifying specific characters of larval stages. Cook (1966b) reared larvae of *P. aztecus* and *P. duorarum*, but could not detect a difference in either setation or ratios of various morphological features. Subrahmanyam (1971) studied larvae taken from the plankton, and although he could separate two species of *Sicyonia* using setation on the 2nd antenna endopod, he was unable to subdivide three species of *Penaeus* (*P. fluviatilis* [= *P. setiferous*], *P. aztecus, P. duorarum*) or two species of *Trachypenaeus* (*T. similis* and *T. constrictus*) on the basis of morphology or morphometry. He suggested that studies at the biochemical or molecular level may be necessary to achieve this. Cook and Murphy (1971) reared *P. aztecus, P. duorarum* and *P. setiferus* and found them to be identical in setation and other major morphological characteristics. They also searched unsuccessfully for differences in various morphological ratios. Courties (1976) reared *P. indicus* and *P. semisulcatus* to 1st zoea, and could not distinguish them. Most recently, Motoh (1979) and Motoh and Buri (1979) reared larvae of *P. monodon* and *P. merguiensis* and compared them with *P. japonicus* described by Hudinaga (1942). They listed several differences between the species, chiefly the nature of the supraorbital spine in 2nd zoea, and the segmentation and setation of the 2nd maxilliped in several stages. However, they mentioned that these differences might well be due to individual or local variation, or to the effects of different rearing conditions. In support of this idea, Motoh and Buri (1979) drew attention to several differences in the description of the larvae of *P. merguiensis* raised by them and the same species raised by Raje and Ranade (1972). Many of the characteristics of the early larval stages are subject to variation induced by growth during an instar (Hudinaga, 1942; Motoh, 1979; Motoh and Buri, 1979) as well as the possible effects of environmental variability.

We therefore decided to seek a statistical method for identifying larvae which would take into account the variability in larval characters, and would use a number of characters simultaneously. Although the clustering multivariate techniques of numerical taxonomy have been used to identify unknown animals by pooling the morphological data for both knowns and unknowns and applying a clustering program to this data matrix (*e.g.*, Campbell, 1973; Colwell *et al.*, 1973), this approach is most useful when the aim is to study the nature of the taxonomic boundaries involved. When the problem is solely identification, discriminant analysis is the preferred technique. A full treatment of the theory and application of discriminant analysis is given in Lachenbruch (1975).

In this paper we describe the methods used to obtain reference specimens of penaeid larvae and test the discriminant analysis with the reference material. We then apply the technique to the first zoeal (= protozoeal) larvae from plankton samples obtained on one cruise in the Gulf of Carpentaria, Australia in March, 1977.

Materials and Methods

Ovigerous females—collection and spawning

Ovigerous females were sought on commercial shrimp grounds, using chartered trawlers. Ripe and healthy females were sorted from the catch, as quickly as possible, and placed in clean sea water which was obtained by bucketting. Sea water from deck hoses was avoided because of elevated temperatures and the possibility of metallic or other contaminants in the ship's plumbing. After the whole catch had

been sorted the females that had been chosen were re-examined. Over the period of sorting, weak and moribund animals became more obvious. Vigorous heartbeat and action of the scaphognathite were the primary criteria for health. The ripeness of the ovaries was assessed subjectively. This assessment was usually a combination of depth of color, granular appearance, and degree of fullness as estimated by the size of the ovarian outpockets on the dorsal side of the first abdominal segment. Females meeting these criteria were placed in the holding tanks (see section under larval rearing). Through the night's trawling, females were monitored at approximately half-hour intervals for health and spawning. Culling was continuous, the ripest females from each trawl replacing less ripe animals. Careful selection of ripe females resulted in a high proportion of spontaneous spawnings; induction methods were not found to be necessary. Normally spawning was extremely rapid and was manifested by a coat of bubbles on the top of the holding container and a thick band of pink proteinaceous foam around the edge of the container at the water line. The presence of eggs was verified by dipping from the container with a clear beaker. The eggs were usually left for about one half hour so that they would be sufficiently robust for subsequent handling, and then siphoned from the holding containers successively through a 500-μm screen which retained most fecal matter and other debris, and a submerged 90-μm screen which retained the eggs. They were washed several times before being pipetted into larval rearing containers or egg transport modules.

Shipboard larval rearing

Because of the limited space on chartered trawlers, a rack capable of holding four tiered 600 mm square PVC baths of running sea water, was constructed of bolted channel section galvanized steel (Fig. 1;B). This portable apparatus could be dismantled and shipped by air to remote areas and moved easily from a vessel to a shorebased rearing facility. The top three water baths, used for larval culture (Fig. 1;1, 2, 3), each contained 36 tall-form 400 ml beakers, containing 250 ml of sea water. The fourth water bath (Fig. 1;4) was used for brine shrimp culture, and held 16 one litre beakers, each containing 700 ml of sea water. A fifth tray, without flowing sea water, held 36 spare 400 ml beakers. All beakers had glass petri dish lids to prevent splash contamination. The water level in each tray was maintained by a weir at a level slightly below the water level within the beakers. Water flow (3 l/min) was directed through the tray and past each beaker, in series, by baffles. This flow rate was sufficient to prevent any noticeable temperature differential across the water bath and the baffles prevented water surge as the boat rolled.

Surface sea water was supplied by a 25 mm centrifugal electric pump (Fig. 1;C). Water flow was controlled by a manifold of 6 valves, four controlling the larval water baths and two regulating the surplus water which was used to cool the female holding tanks (Fig. 1;D). Aeration to the rack was provided by a 4-diaphragm aquarium aerator (Fig. 1;A). The manifold for each tray was supplied by a separate diaphragm, in order to localize the effects of a diaphragm failure. Air supply to individual larval rearing beakers was regulated at 35 ml/min by a 230 mm length of 0.38 mm ID polyethylene tubing attached to the rigid 25 mm diameter PVC air manifold. Each beaker's aerator was weighted with a section of thick walled glass capillary tubing. The airflow to the brine shrimp beakers was regulated at 60 ml/min by a 150 mm length of the 0.38 mm ID tubing.

Larvae were fed a mixture of three species of marine phytoplankton (*Thalas-*

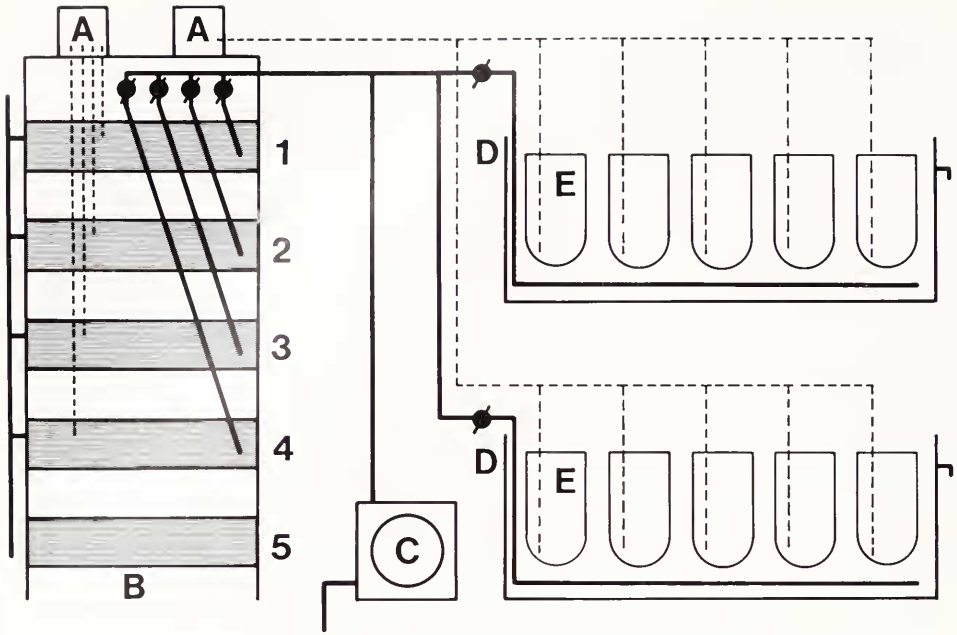

FIGURE 1. Shipboard larval rearing rack (B), with three water baths (1, 2, 3) for larval rearing, one (4) for rearing brine shrimp and storage (5) for spare larval rearing beakers. Female isolation chambers (E) in circulating water baths (D). Pump (C) to supply surface sea water for temperature regulation of both the larval incubation trays and the female holding containers. Two aerators (A) to supply air to individual larval and adult containers. See text for details of construction and operation.

siosira pseudonana, Isochrysis galbana, Tetraselmis (= Platymonas) suecica) that previously had been grown, harvested, concentrated to predetermined cell concentrations, and frozen with dimethyl sulfoxide (DMSO) as a freeze-thaw protectant (Brown, 1972) in 10 ml plastic test tubes (Pendrey, unpublished). The algae were thawed and diluted to pre-determined feeding aliquots, on board, and fed to the larval cultures four times per day. Brine shrimp cultures were maintained on board for feeding to the older larvae (mysis and postlarvae) along with the algal diet.

The sides of the rack were covered by 70% shade cloth to keep the cultures cool and prevent direct sunlight entering the larval containers. The top of the rack had a pitched waterproof canvas roof to protect the aerators from rain and salt spray.

Female-holding and spawning containers

Two large fiberglass tanks (500 × 1200 × 600 mm) (Fig. 1;D) with flow-through sea water circulation were used as water baths for maintaining gravid females at surface sea water temperatures. The females were held in 9 l polyethylene bags (Fig. 1;E) (10 bags per water bath) held open with a polyethylene ring and suspended from a metal rack across the top of the water bath. A separate aquarium aerator was used for these female holding tanks. Airflow to each plastic bag was regulated by a 25 mm length of 0.38 mm ID polyethylene tubing. The standing water in each bag was treated with 0.1 ppm EDTA to suppress bacterial growth. Each bag could hold up to five large females of the same species. The female-holding tanks were

covered with waterproof canvas to exclude light, rain and fall-out from the ship's diesel exhaust.

Egg transport modules

Under certain circumstances shipboard rearing was not desirable. A method of transporting eggs from spawning locations to our laboratory was developed for these instances. Self-contained modules (Fig. 2) enabled us to airfreight eggs or early larvae. The modules were immersed in 12–16 l of sea water in an insulated polyethylene container. Air was provided by a battery operated aerator which would run for more than 48 hours on one set of batteries. An air lift forced the water through a 200 g bed of coarse activated charcoal to remove any organic toxins. The water then passed through a 20 mm thickness of Dacron wool to remove detritus and then through a 142-μm nylon mesh which supported the eggs. The water flowing up through the eggs gently agitated and aerated them and prevented dense anaerobic clusters from forming. Any naupliar stages that hatched in transit were retained in the incubation chamber formed by a clear 90 mm diameter acrylic plastic tube and a removable 142-μm mesh cap. The whole unit was immersed so that water could flow unimpeded over the rim of the cap. The pipe to release the air from the airlift was about 40 mm above the water surface. The use of 0.1 ppm EDTA during egg transport in some cases seemed to lead to higher hatching rates.

Larval morphology

Larvae for our reference collection were obtained both from our own shipboard and laboratory rearing, and from other sources (see Results, Reference collection section). Samples of larvae for morphological studies were preserved on several occasions during each larval instar to ensure that the full range of morphological variability for a given instar would be represented.

Larvae were preserved initially in buffered (sodium tetraborate) 10% formaldehyde. They were then transferred via 70% ethanol to a PVA/phenol/lactic acid medium with chlorazol black E (Perkins, 1956) at least two days before any morphological measurements were made. Individual larvae were placed in a drop of PVA medium, on a well slide, without a cover slip. The viscosity of the PVA medium allowed the larva to be arranged easily for subsequent viewing and measurement. Measurements were made using a compound microscope, with 10× ocular equipped with micrometer, and a 10× phase contrast objective. All measurements were recorded to the nearest 0.1 ocular division (1.0 ocular division = 0.118 mm) and all data are presented in ocular divisions (O.D.). Morphological measurements were recorded for a total of 846 1st zoea of four *Penaeus* species (133 *P. merguiensis*, 192 *P. esculentus*, 355 *P. semisulcatus*, and 166 *P. latisulcatus*) from the reference collection. The 14 measurements made (Fig. 3) were: 1) Total length; 2) Carapace length; 3) 1st Antenna length; 4, 5, 6) Lengths of segments of 1st antenna; 7) Diameter of 2nd antenna basis; 8) Length of 2nd antenna endopod; 9) Length of 2nd antenna exopod; 10, 11, 12) Lengths of segments of 2nd antenna endopod; 13) Diameter of abdomen; 14) Diameter of 1st antenna.

Discriminant analysis was performed on the CYBER 76 computer operated by CSIRO Division of Computing Research, Canberra, Australia; the DISCRIMINANT subprogram of the Statistical Package for the Social Sciences (SPSS) software library (Nie *et al.*, 1975) was used. We developed interactive programs for data collection and identification of unknown larvae. These programs were run on a PDP-11 minicomputer.

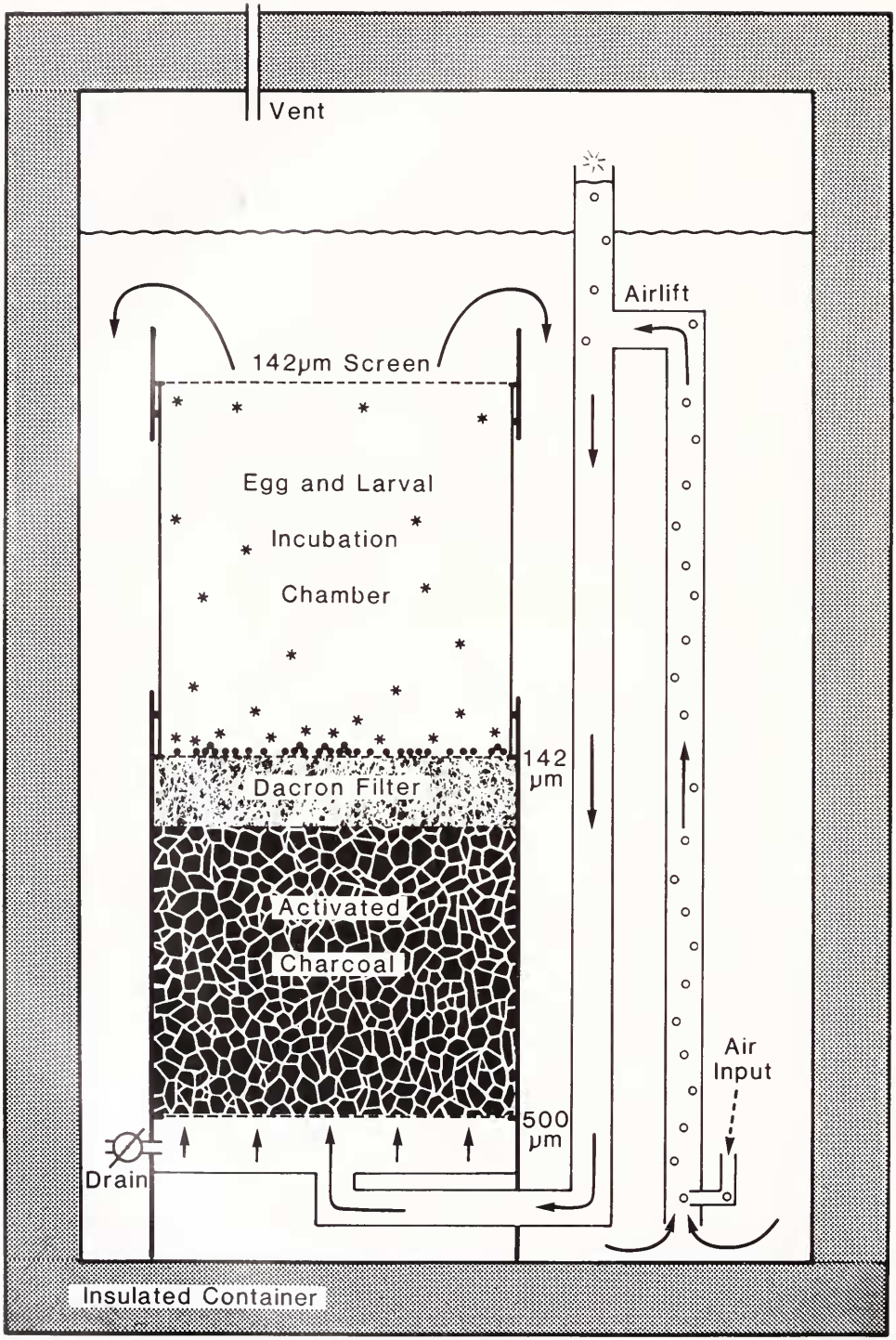

FIGURE 2. Egg and larval transport modules in an insulated water container. Figure not drawn to scale. See text for details of construction and operation.

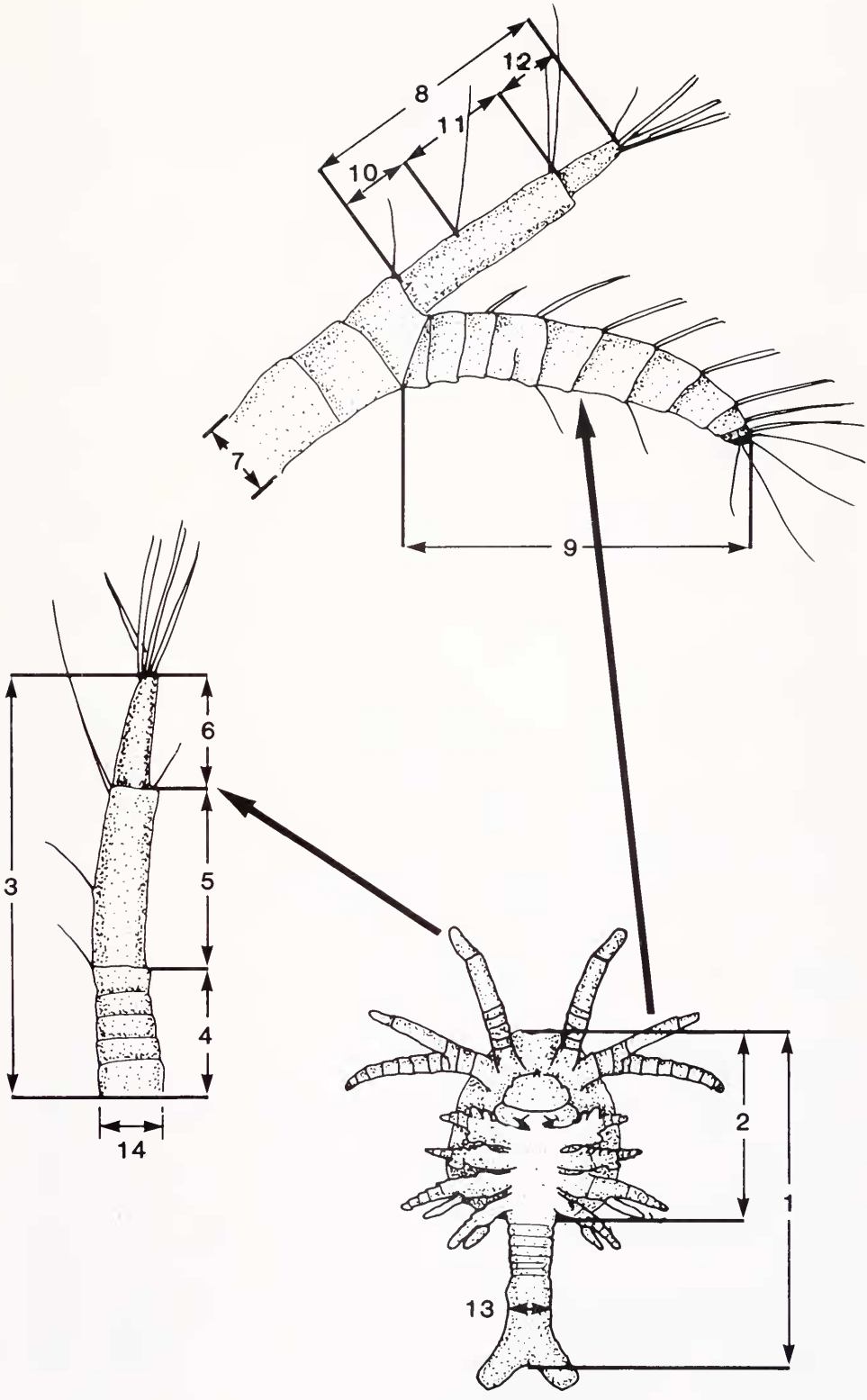

FIGURE 3. Fourteen morphological characters of the first zoea penaeid larva used in discriminant analysis.

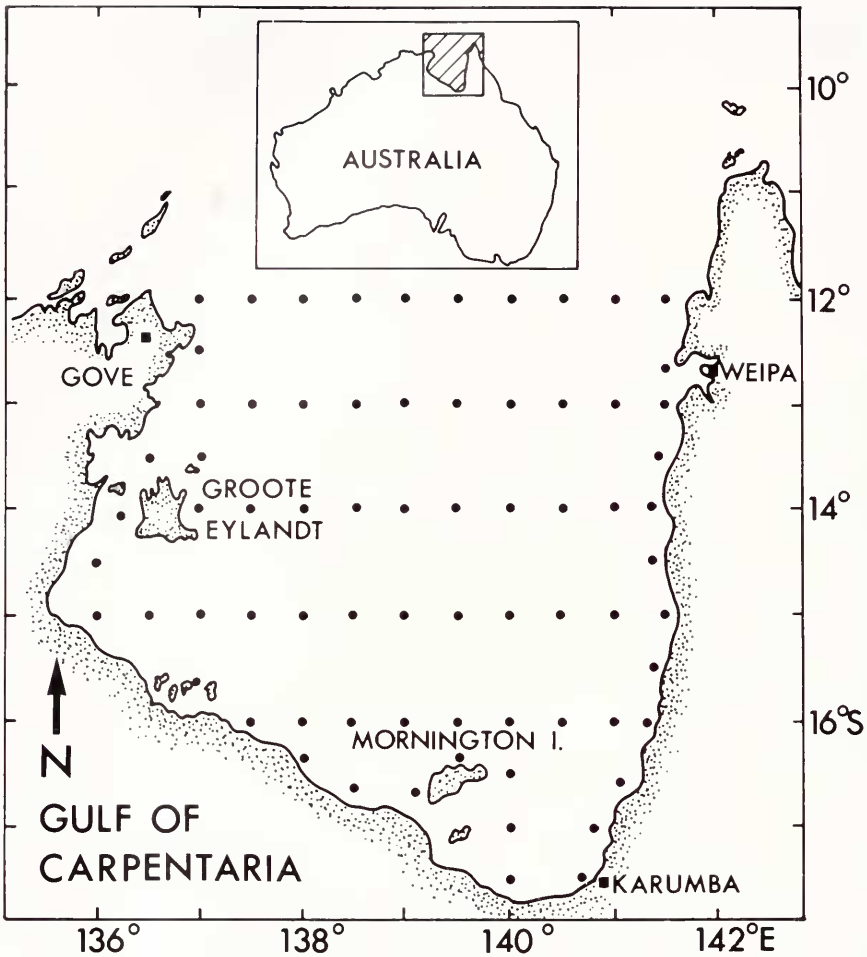

FIGURE 4. Gulf of Carpentaria, Australia. Dots indicate location of plankton samples taken on cruise in March, 1977.

Plankton sampling

Plankton samples were obtained in a manner described by Rothlisberg and Jackson (1982). Paired samples (142 and 500 μm nets), analyzed in this study, were taken between 16 and 30 March 1977 at 70 stations (Fig. 4).

All samples were initially split in half using a Folsom plankton splitter (McEwen *et al.*, 1954). One half of the formalin-fixed sample was subjected to a destructive biomass analysis (Rothlisberg and Jackson, 1982) while the other half was split further, transferred to 2% 2-phenoxyethanol and stored for microscopic analysis. Larvae from the 142 μm samples were sorted from these fractions using binocular dissecting microscopes. Larval numbers were standardized by calculating the volume of water filtered, from calibrated flowmeter readings, and then multiplied by the sample depth, from a time-depth recorder trace, to give larval abundance values integrated under 1 m^2 of sea surface (Kramer *et al.*, 1972).

TABLE I

Species and stage of development of penaeid larvae in reference collection

	Larval stage of development							
		Zoea			Mysis			
Species	Nauplius	1	2	3	1	2	3	Postlarva
Penaeus								
P. esculentus	*	*	*	*	*	*	*	*
P. indicus	*	*	*	*	*	*	*	*
P. latisulcatus	*	*	*	*	*	*	*	*
P. merguiensis	*	*	*	*	*	*	*	*
P. semisulcatus	*	*	*	*	*	*	*	*
Metapenaeus								
M. bennettae	*	*	*					
M. eboracensis	*	*	*	*				
M. endeavouri	*	*	*	*	*	*	*	*
M. ensis	*	*	*	*	*	*	*	*
M. insolitus	*	*	*	*	*			
Trachypenaeus								
T. anchoralis	*	*	*	*	*	*	*	*
T. fulvus	*	*	*	*	*	*		
Atypopenaeus								
A. formosus	*	*	*	*	*			
Parapenaeopsis								
P. cornuta	*	*	*	*				
Metapenaeopsis								
M. novaeguinea	*	*	*	*	*			
M. palmensis	*	*	*	*	*	*	*	*
Genera	6	6	6	6	5	4	4	4
Species	16	16	16	15	13	10	9	9

RESULTS

Larval reference collection

Table I contains the species and stage of larval development we have obtained for our reference collection. All but a few of the species were obtained in the Gulf of Carpentaria using the shipboard rearing facilities. *Penaeus indicus* was spawned in the Gulf of Papua, off Yule Island, and the eggs were shipped to our laboratory in Cleveland, southern Queensland. We have also spawned *P. merguiensis, P. esculentus,* and *Metapenaeus bennettae* on chartered trawlers in Moreton Bay, adjacent to our laboratory. These *P. merguiensis* and *P. esculentus* were used to compare with and supplement the number of larvae we had obtained by shipboard spawning and rearing in the Gulf of Carpentaria. Specimens of *P. merguiensis* have also been obtained from France-Aquaculture, New Caledonia.

In order to enlarge our collection of *P. semisulcatus* larvae we obtained ovigerous females off Cairns, northern Queensland. Unlike the Gulf of Carpentaria cruises during which *P. semisulcatus* was spawned routinely, we had repeated difficulty obtaining viable eggs on board. The ripe ovaries frequently would change color,

TABLE II

Mean, standard deviation and range of 14 morphological characters for the first zoeal instar of four species of Penaeus

Character	*Penaeus esculentus* (n = 192)	*Penaeus latisulcatus* (n = 166)	*Penaeus merguiensis* (n = 133)	*Penaeus semisulcatus* (n = 355)
Total Length (1)*	8.55 (1.14) 6.1–11.0	7.68 (0.85) 5.7–9.4	7.63 (0.78) 5.9–9.6	8.09 (0.84) 5.6–10.1
Carapace Length (2)	3.88 (0.34) 3.0–4.5	3.53 (0.33) 2.6–4.1	3.71 (0.29) 3.0–4.3	3.94 (0.34) 2.7–4.6
First Antenna Length (3)	3.16 (0.13) 2.8–3.4	3.58 (0.16) 3.1–4.0	2.94 (0.17) 2.5–4.1	3.25 (0.21) 2.5–3.6
First Antenna Segment 1 (4)	1.00 (0.09) 0.8–1.2	1.15 (0.11) 0.8–1.4	0.94 (0.10) 0.7–1.3	1.08 (0.10) 0.8–1.4
First Antenna Segment 2 (5)	1.40 (0.08) 1.2–1.6	1.57 (0.10) 1.3–1.8	1.24 (0.08) 1.0–1.4	1.39 (0.11) 1.0–1.6
First Antenna Segment 3 (6)	0.79 (0.06) 0.6–0.9	0.88 (0.07) 0.7–1.0	0.77 (0.07) 0.6–0.9	0.82 (0.08) 0.5–1.0
Diameter of Second Antenna (7)	0.54 (0.09) 0.3–0.7	0.52 (0.06) 0.3–0.7	0.50 (0.08) 0.3–0.6	0.52 (0.09) 0.2–0.7
Second Antenna Endopod Length (8)	1.90 (0.09) 1.5–2.1	2.22 (0.11) 2.0–2.5	1.78 (0.12) 1.6–2.8	2.03 (0.13) 1.7–2.5
Second Antenna Exopod Length (9)	2.07 (0.15) 1.6–2.5	2.48 (0.20) 1.9–3.0	1.99 (0.13) 1.6–2.3	2.20 (0.19) 1.4–2.7
Second Antenna Endopod Segment 1 Length (10)	0.45 (0.06) 0.3–0.8	0.66 (0.08) 0.5–0.8	0.40 (0.05) 0.3–0.5	0.53 (0.06) 0.4–0.7
Second Antenna Endopod Segment 2 Length (11)	0.91 (0.07) 0.7–1.0	1.00 (0.07) 0.8–1.1	0.89 (0.07) 0.7–1.0	1.00 (0.08) 0.7–1.2
Second Antenna Endopod Segment 3 Length (12)	0.55 (0.06) 0.4–0.6	0.58 (0.05) 0.5–0.7	0.50 (0.05) 0.4–0.6	0.53 (0.06) 0.3–0.7
Abdomen Diameter (13)	0.90 (0.16) 0.5–1.2	0.87 (0.12) 0.6–1.1	0.84 (0.13) 0.5–1.2	0.95 (0.16) 0.3–1.3
First Antenna Diameter (14)	0.52 (0.08) 0.2–0.7	0.45 (0.07) 0.2–0.6	0.44 (0.07) 0.3–0.6	0.48 (0.10) 0.1–1.2

* Morphological character number—Figure 3 and text.
Measurements are in ocular divisions (1 O.D. = 0.118 mm).

from the normal deep blue-green to grey, within minutes of capture. Enomoto (1971) reported a similar occurrence for *P. semisulcatus* in Kuwait. This color change was often accompanied by partial spawnings of non-viable eggs. This phenomenon was observed over four cruises in the period November 1980 to May 1981 off Cairns. Viable eggs were finally obtained following shipment of ripe females to our laboratory and inducement by eyestalk ablation.

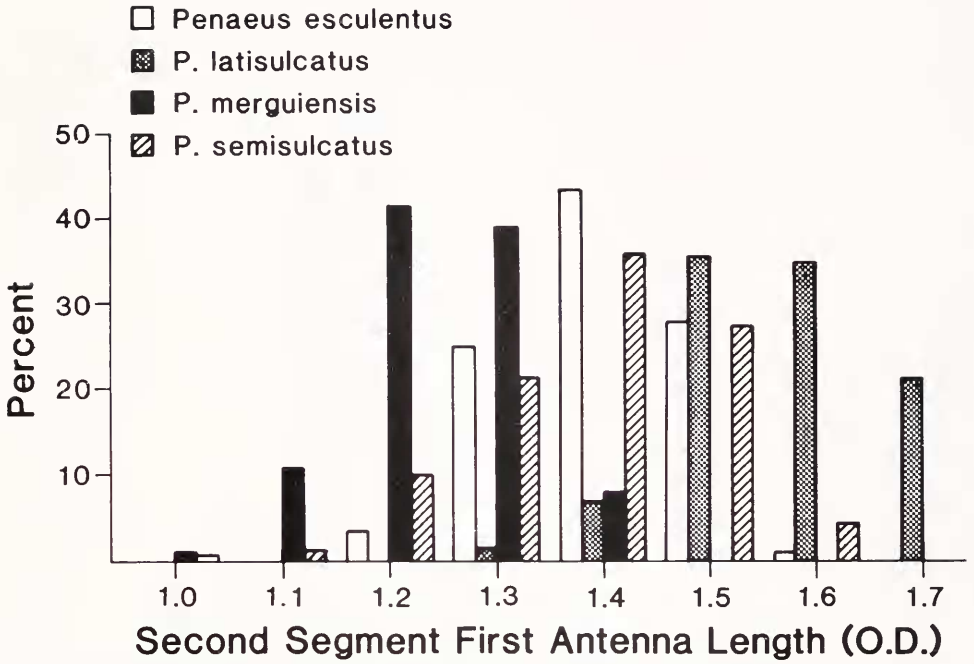

FIGURE 5. Frequency distribution of the length of the second segment of the first antenna of first zoeal instar for four species: *Penaeus esculentus; P. latisulcatus; P. merguiensis;* and *P. semisulcatus.* Ocular division (O.D.) = 0.118 mm.

Though *P. latisulcatus* occurs in the Gulf of Carpentaria we have never caught a suitably ripe female. The larvae in our reference collection were obtained from a commercial prawn farm in Port Broughton, South Australia, on two separate occasions.

Our reference collection (Table I) now includes 15 (*M. bennettae* does not occur in the Gulf) of the 37 species of penaeids reported from the Gulf of Carpentaria and surrounding waters (Dall, pers. comm.). More importantly, we have complete larval series for the four numerically dominant commercial species in the genus *Penaeus: P. esculentus, P. merguiensis, P. latisulcatus,* and *P. semisulcatus.* Four other species (*P. indicus, P. japonicus, P. longistylus,* and *P. monodon*) are known to occur in the Gulf, but at present are numerically insignificant.

Larval morphology

An initial examination of the morphological data for the four *Penaeus* species revealed that, although there was considerable overlap, most characters showed differences between the species (Table II). The range in size and the overlap in distributions between species for the second segment of the first antenna is an example (Fig. 5). When larvae sampled within a few hours of the molt to 1st zoea are compared with larvae sampled toward the end of that instar, it is apparent that some of the characters (*e.g.,* total length, Fig. 6A) undergo considerable change during the intra-molt period. However, other characters (*e.g.,* length of first antenna, Fig. 6B) exhibit less of this type of variation. Therefore, any method of identifying early larval penaeids must allow for considerable variation in morphology.

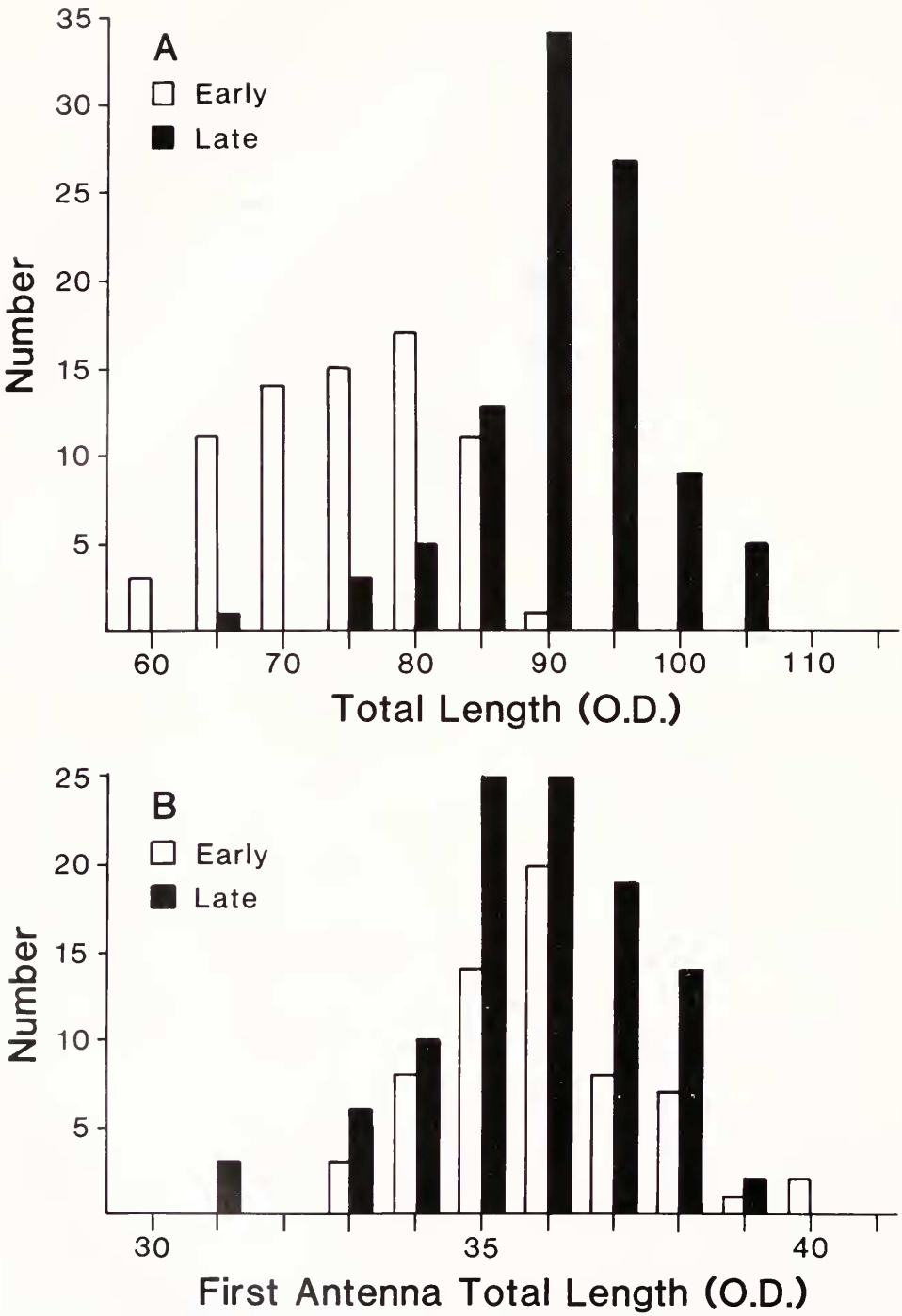

FIGURE 6. A. Frequency distributions of total length indicating growth of this character during the first zoeal instar of *Penaeus latisulcatus*. B. Absence of growth in the length of the first antenna during the first zoeal instar of *P. latisulcatus*. Ocular division (O.D.) = 0.118 mm.

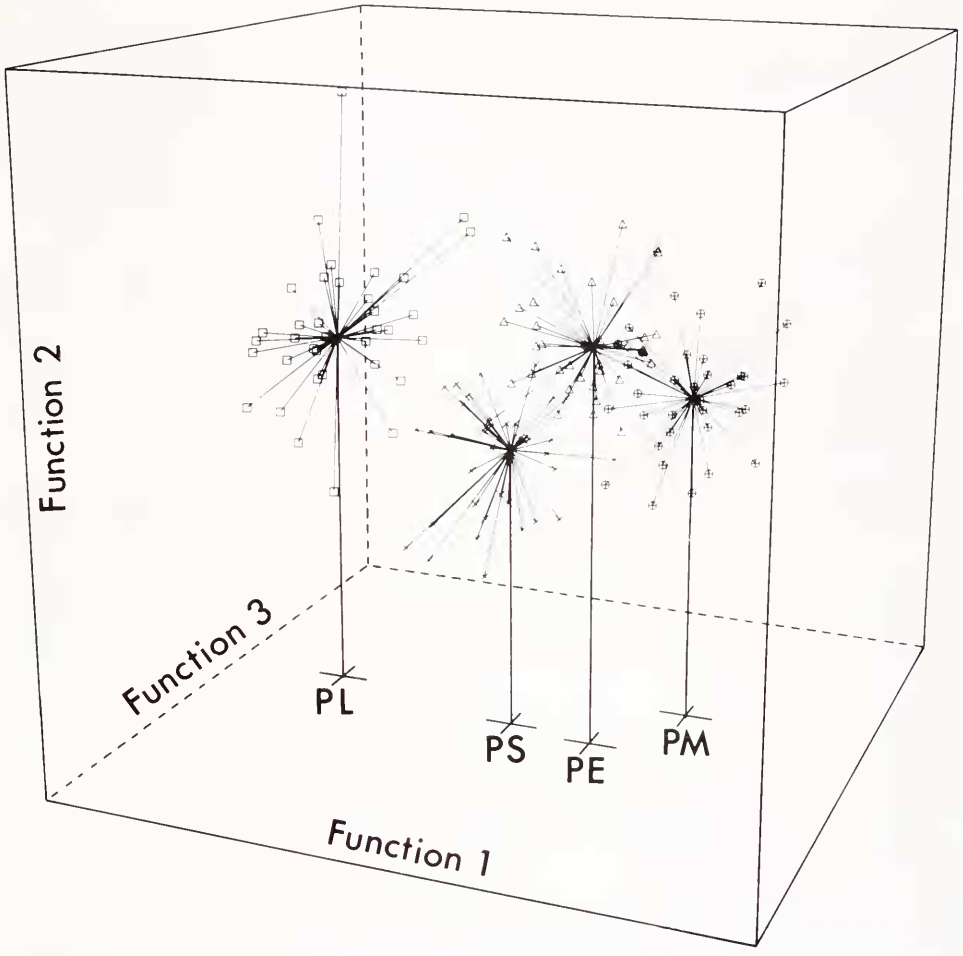

FIGURE 7. Three dimensional separation of four species: *Penaeus esculentus* (PE); *P. latisulcatus* (PL); *P. merguiensis* (PM); *P. semisulcatus* (PS) using three discriminant functions.

Larval identification

A stepwise discriminant analysis applied to the data for the four *Penaeus* species produced three discriminant functions which, when used to classify the reference collection larvae (as if their identity was unknown) resulted in 87.3% overall correct classification. Characters 6 and 7 (Fig. 3) however, did not contribute significantly to this separation, and the information provided by characters 4 and 13, although significant, was small. Characters 3 and 8, although providing useful information, were not necessary since the same information was provided by 4, 5 and 6 in the case of 3, and by 10, 11 and 12 in the case of 8. Therefore a second discriminant analysis was performed with characters 3, 4, 6, 7, 8 and 13 excluded. The resulting 8 characters produced 3 discriminant functions which correctly classified 85.4% of the larvae. The amount of three-dimensional separation achieved according to their score on each of the 3 discriminant functions is shown in Figure 7.

TABLE III

Effect of criterion value (see text for definition) on accuracy of larval identification of four species of Penaeus

	Criterion value							
	1.000	1.001	1.002	1.003	1.004	1.005	1.006	1.007
P. esculentus								
Percent correctly identified*	80.8	83.6	85.4	86.8	88.9	91.1	92.2	93.6
Percent unidentifiable**	0.0	5.6	10.6	15.5	21.7	30.4	36.6	41.6
P. latisulcatus								
Percent correctly identified	95.6	95.6	95.6	97.0	97.0	97.7	97.7	98.4
Percent unidentifiable	0.0	0.0	0.0	1.5	2.2	3.7	3.7	4.4
P. merguiensis								
Percent correctly identified	89.1	91.3	92.1	94.7	95.6	97.7	98.8	98.8
Percent unidentifiable	0.0	5.5	8.2	13.6	17.3	21.8	23.6	26.4
P. semisulcatus								
Percent correctly identified	81.5	83.7	85.0	86.1	88.9	89.3	89.2	90.8
Percent unidentifiable	0.0	4.8	8.5	12.2	16.7	20.4	28.2	31.5
Overall								
Percent correctly identified	85.4	87.4	88.6	90.1	91.8	93.0	93.6	94.7
Percent unidentifiable	0.0	4.1	7.2	11.1	15.0	19.6	24.5	27.6

* Percent of the number of larvae that were above the criterion level and were correctly identified.

** Percent of the number of larvae below the criterion level, and therefore identification was uncertain (see text).

The SPSS Discriminant program provides an easy method for identifying unknown larvae. The discriminant functions are used to generate a set of classification functions. In this case there are four classification functions, one corresponding to each of the four *Penaeus* species. An unknown larva can be identified by using it's eight measurements to calculate four scores, one for each of the four functions. The unknown is then classified according to the highest score. In this study, in order to increase the accuracy of identification, for each unknown the ratio between the two highest classification scores was examined. If this ratio (= criterion) fell below a certain level the identification reverted to unidentified, on the basis that the initial identification was relatively uncertain. The effect of varying this criterion value when the larvae of the reference collection were identified in this way is shown in Table III. The proportion of correct identifications increased from 85.4%, when all identifications were accepted, to 94.7% when the test was severe enough to classify 27.6% of doubtful larvae as unidentified. At all criterion levels *P. latisulcatus* was readily separated from the other three species, having a high percentage of correct classifications and the lowest proportion remaining unidentified. On the other hand, the two tiger prawns *P. esculentus* and *P. semisulcatus* were the most similar: adults of these species are also difficult to distinguish. As a compromise between increasing the proportion of correct identifications, and reducing the amount of lost information due to larvae being classified as unknown, an operational criterion value of 1.003 was chosen. This produced overall 90.1% correct identifications, while 11.1%

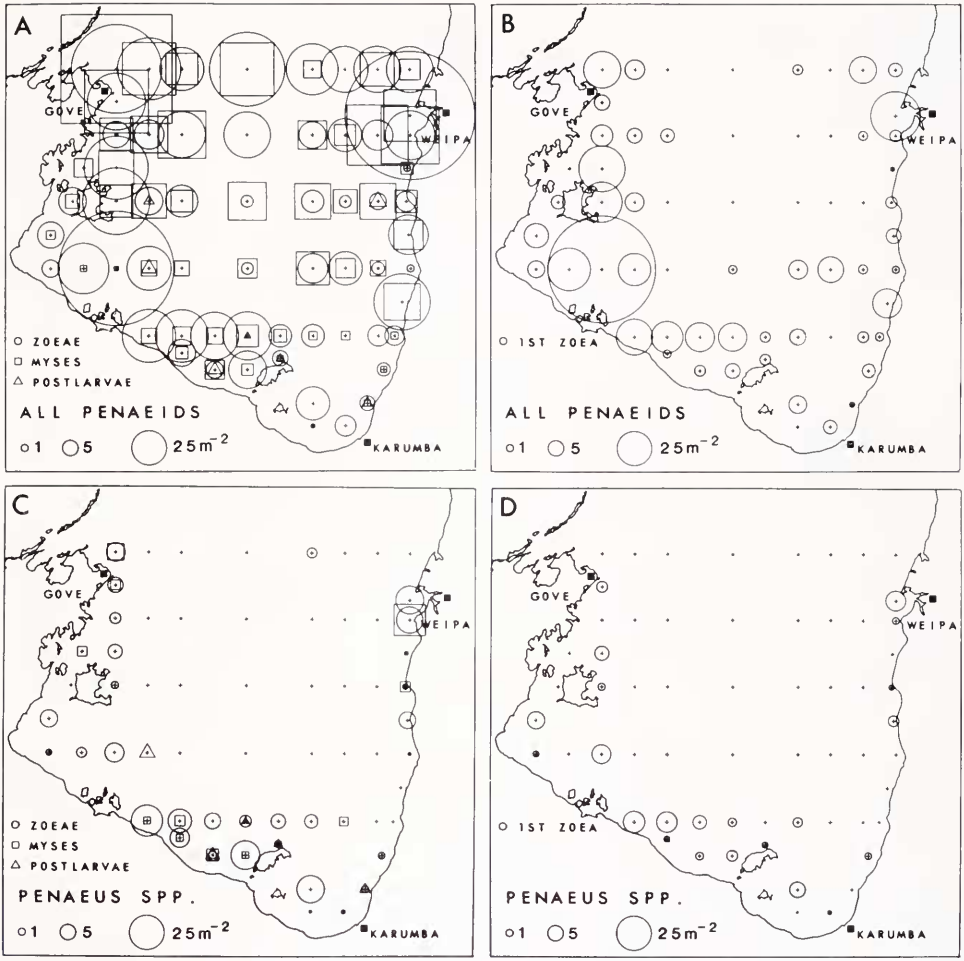

FIGURE 8. Larval distribution and abundance in the Gulf of Carpentaria in March 1977. A. Three zoeal and three mysis larval substages and postlarvae of all species of penaeids. B. First zoeal larval substage of all species of penaeids. C. Three zoeal and three mysis larval substages and postlarvae of all species in the genus *Penaeus*. D. First zoeal larval substage of all species in the genus *Penaeus*.

remained unidentified. At this level the two species of tiger prawn were accurately identified more than 86% of the time.

An interactive computer program was written to identify larvae routinely in this way. A technician, using a computer terminal, is prompted for values for the eight measurements, and these are queried by the program if they fall outside the normal range. Classification scores are calculated, and the ratio between the two highest scores is compared with a criterion of 1.003; the resulting identification is then reported to the operator. Identifications, together with all character measurements, are stored on floppy discs for later use.

Larval distribution and abundance

Penaeid larvae, potentially from almost 40 species, were found in most samples taken in the Gulf of Carpentaria in March 1977 (Fig. 8A). First zoea larvae appear

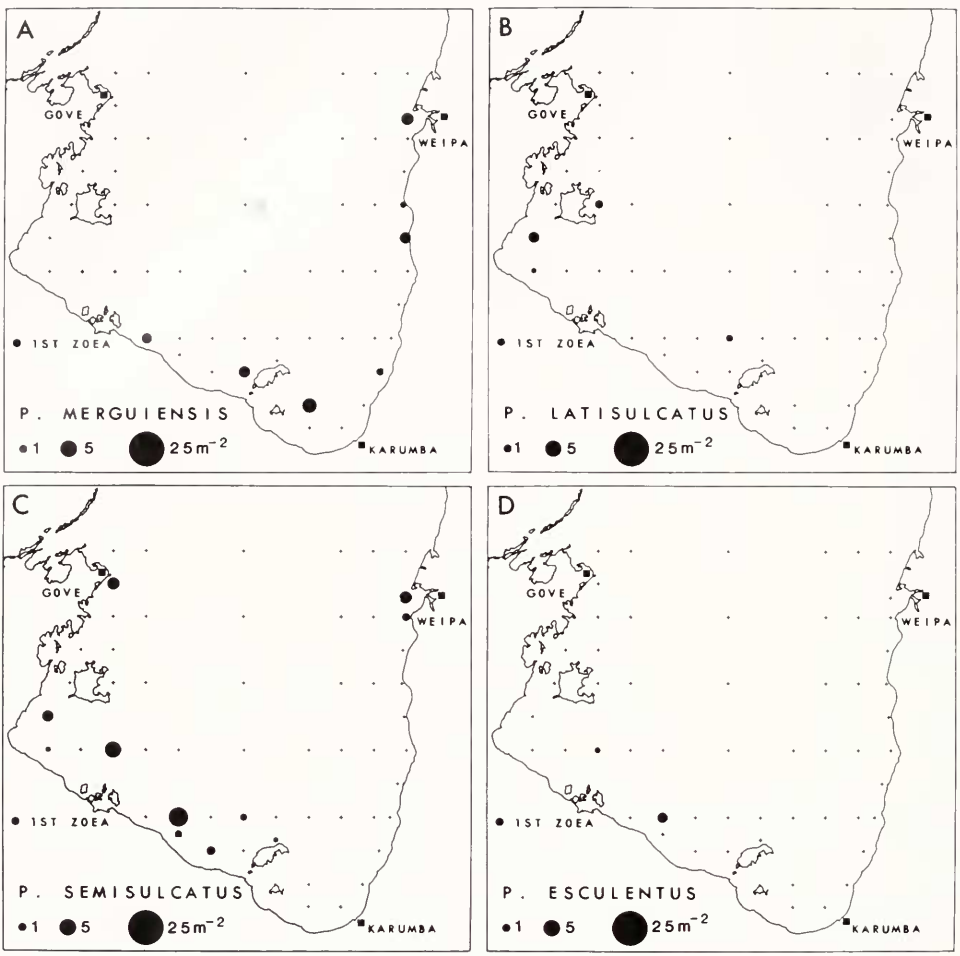

FIGURE 9. Distribution and abundance of the first zoeal instar of four species of *Penaeus* in the Gulf of Carpentaria, Australia in March 1977. Identification of species by discriminant function method, using criterion = 1.003. See text for details of method. A. *Penaeus merguiensis*. B. *P. latisulcatus*. C. *P. semisulcatus*. D. *P. esculentus*.

approximately 48 hours after spawning and their presence is a good indication of spawning activity; for this reason it seemed unnecessary to attempt the difficult task of sampling nauplii quantitatively and identifying them for the sake of marginally higher resolution of spawning time and location. The distribution of first zoeae indicated that penaeid spawning was widespread and abundant (Fig. 8B).

Penaeus larvae and postlarvae were restricted to a relatively narrow coastal band (Fig. 8C). Thus the large numbers of early penaeid zoeae found in much of the Gulf of Carpentaria (Fig. 8B) were not *Penaeus* species (Fig. 8D).

With application of the discriminant analysis, specific resolution of the first zoeal distributions was obtained (Fig. 9). The distribution of *P. merguiensis* larvae (Fig. 9A) was discontinuous and restricted to three areas of the Gulf; Albatross Bay, off Weipa in the north eastern Gulf; along the middle eastern side of the Gulf; and the southern Gulf, both east and west of Mornington Island. Early larvae of *P. latisul-*

catus (Fig. 9B) were absent from the eastern side of the Gulf and were most abundant around Groote Eylandt. First zoeae of *P. semisulcatus* (Fig. 9C) were the most widespread of the four species as spawning occurred in Albatross Bay on the north eastern side of the Gulf and over an extended area on the western side, from the Gove peninsula, south of Groote Eylandt, down to west of Mornington Island. *Penaeus esculentus*, though very abundant as adult shrimp at this time, did not appear to be spawning (Fig. 9D).

The decreased number of larvae represented in Figure 9, compared with Figure 8D, was due either to the presence of larvae of species other than the four revealed by the identification procedure, or to larvae of those four species, but with morphology falling outside the acceptable limits set by our criterion values.

Discussion

In temperate penaeid fisheries, where diversity is relatively low and there may be temporal and spatial separation of reproductive activity by species, larvae in the plankton can be 'identified' on a probability basis with some assurance, given knowledge of adult distribution, abundance and reproductive state (Munro *et al.*, 1968; Roessler *et al.*, 1969; Jones *et al.*, 1970). In tropical penaeid fisheries which are characterized by highly diverse genus and species assemblages, there may be little spatial or temporal separation of their prolonged reproductive activity. This is particularly true in the Indo-West Pacific, including the Gulf of Carpentaria. Here it is not possible to guess the identity of larvae by examining the temporo-spatial distribution of spawning adults, and the larvae must be identified with a robust taxonomic method.

Establishing a reference collection of penaeid larvae has been the first step in enabling identification of larvae from our plankton collections. The shipboard rearing technique we developed was extremely useful, allowing us to spawn adults and rear larvae in remote areas with no laboratory facilities. Shipment of ovigerous females or eggs from these locations would not have been practicable.

Given the high degree of variation in morphology within species, as well as that due to growth within instars, the few larval descriptions in the literature were of little value because they were based on small numbers of specimens and did not report characters which had taxonomic value at the species level. A collection with large numbers of larvae, sampled at frequent intervals from diverse sources, was necessary.

Even with a large amount of larval material at hand however, it became obvious that simple, single character comparisons could not be used for separating penaeid larvae because of the large degree of overlap in both merisitic and morphometric characters. A technique had to be found that was practicable for screening large numbers of larvae from field collections. Characters were useful only if they could be measured with a minimum of handling and no dissection. Discriminant analysis, a multivariate technique developed by Fisher (1936), sums the information provided by a number of quantitative values, each of which shows incomplete, but significant differences between species. Although this is the first time the technique has been applied to larval crustacean taxonomy, it has been applied to a variety of taxonomic problems, for example: Africanized honeybee hybrids (Daly and Balling, 1978); pink salmon (Pearson, 1964); human crania (Giles and Elliot, 1962, 1963); caddis larvae (Buholzer, 1977); nematode eggs (Lysek *et al.*, 1975); and adult American lobster populations (Saila and Flowers, 1969).

There are several areas of penaeid larval identification requiring further development. The first is to apply the technique to later zoeal instars and later larval

stages. This involves morphological character assessment on a instar by instar basis. Work is proceeding on experimental larval rearing studies to test the validity of the numerical taxonomic technique, bearing in mind the morphological lability seen in the early larvae. We are also increasing the number of species in our reference collection, not only so we can identify the still unidentified larvae in our samples, but also to see if the technique can be used in other geographic species complexes or broadened to the whole genus *Penaeus*. Work on the genus *Metapenaeus*, which also supports important fisheries, is underway. Finally, having developed a complex interactive procedure of larval examination and identification there is some evidence that a skilled operator, with some experience, can identify some species based on general shape and other subtle characters that are very hard to quantify. If these subjective criteria can be made more objective and repeatable between observers, the time, expense and technology required for larval identification should be lessened and the application broadened.

Our progress with larval identification has begun to resolve the confusion regarding penaeid larval distribution and abundance in the Gulf of Carpentaria. As the rest of the larvae from our survey cruises are analyzed, a better Gulf-wide picture of temporal and spatial reproductive dynamics, for the four most important commercial species at least, will be possible. As the later larval stages are incorporated into the identification scheme, factors affecting larval mortality during this short and probably critical planktonic period can be assessed. Finally, hypotheses and mechanisms regarding postlarval recruitment (Staples, 1979) and larval advection (Rothlisberg, 1982; Rothlisberg *et al.*, in press) can be tested in the field.

Acknowledgments

Assistance in rearing at sea was provided by: J. Salini and T. Wassenberg in the Gulf of Carpentaria; D. Gwyther, S. Frusher and C. Tanakani in the Gulf of Papua; and A. Chamberlain and R. Coles in the Coral Sea. Larval rearing was made possible in Moreton Bay through the good graces of G. Mawhinney, skipper of the F/V Zodiac. Larval specimens of *P. latisulcatus* were generously supplied by J. Racovolis and K. Yasuda; M. Autrand provided additional *P. merguiensis* specimens. Algal inocula and culture advice were given by S. Jeffrey, J. Eyles and J. Stauber. A large number of people have helped with the plankton sorting, principally M. Beamish, H. Keag, G. Peate and D. Savage, W. Dall and B. Hill critically reviewed the manuscript.

LITERATURE CITED

Brown, A. 1972. Experimental techniques for preserving diatoms used as food for larval *Penaeus aztecus*. *Proc. Nat. Shellfish. Assoc.* **62:** 21–25.

Buholzer, H. B. 1977. An application of the linear discriminant function and a multivariate analysis to caddis larvae taxonomy. *Proc. 2nd Int. Symp. on Trichoptera, 1977, Junk, The Hague.* Pp. 149–153.

Campbell, I. 1973. Computer identification of yeasts of the genus *Saccharomyses*. *J. Gen. Microbiol.* **77:** 127–135.

Colwell, R. R., T. E. Lovelace, L. Wan, T. Kaneko, T. Staley, P. K. Chen, and H. Tubiash. 1973. *Vibrio parahaemolyticus*—Isolation, identification, classification, and ecology. *J. Milk Food Technol.* **36:** 202–213.

Cook, H. L. 1966a. A generic key to the protozoean, mysis, and postlarval stages of the littoral Penaeidae of the north western Gulf of Mexico. *U. S. Fish Wildl. Serv. Fish. Bull.* **65:** 437–447.

Cook, H. L. 1966b. Identification and culture of shrimp larvae. *U. S. Fish. Wildl. Serv. Circ.* **246:** 12–13.

Cook, H. L., and M. A. Murphy. 1971. Early developmental stages of the brown shrimp, *Penaeus aztecus*, reared in the laboratory. *U. S. Fish Wildl. Serv. Fish. Bull.* **69:** 223–239.

Courties, C. 1976. Descriptions des premiers stades larvaires de trois crevettes peneides pechees a

Madagascar: *Penaeus indicus, P. semisulcatus, Metapenaeus monoceros. Cah. O.R.S.T.O.M. Ser. Oceanogr.*, **14,** 49–70.

Daly, H. V., and S. S. Balling. 1978. Identification of Africanized honeybees in the western hemisphere by discriminant analysis. *J. Kans. Entomol. Soc.* **51:** 857–869.

Enomoto, Y. 1971. Oceanographic survey and biological study of shrimps in the waters adjacent to the eastern coasts of the state of Kuwait. *Bull. Tokai Reg. Fish. Lab.* **66:** 1–73.

Fisher, R. A. 1936. The use of measurements in taxonomic problems. *Annals of Eugenics* **7:** 179–188.

Garcia, S., and Le Reste. 1981. Life cycles, dynamics, exploitation and management of coastal penaeid shrimp stocks. *F.A.O. Fish. Tech. Pap.* **203:** 215 pp.

Giles, E., and O. Elliot. 1962. Race identification from cranial measurements. *J. Forensic Sci.* **7:** 147–157.

Giles, E., and O. Elliot. 1963. Sex determination by discriminant function analysis of crania. *Am. J. Phys. Anthropol.* **21:** 53–68.

Hassan, H. 1974. A generic key to penaeid larvae of Pakistan. *Agric. Pak.* **25:** 227–236.

Hudinaga, M. 1942. Reproduction, development, and rearing of *Penaeus japonicus* Bate. *Jpn. J. Zool.* **10:** 305–393.

Jones, A. C., D. E. Dimitriou, J. J. Ewald, and J. H. Tweedy. 1970. Distribution of early developmental stages of pink shrimp, *Penaeus duorarum*, in Florida waters. *Bull. Mar. Sci.* **20:** 634–661.

Kirkegaard, I. 1975. Observations on penaeid larvae around Australia. Pp. 54–59 in *First Australian National Prawn Seminar, Maroochydore, Queensland, 22–27 November 1973.* P. C. Young, ed. Australian Government Publishing Service, Canberra.

Kramer, D., M. J. Kalin, E. G. Stevens, J. R. Thrailkill, and J. R. Zweifel. 1972. Collecting and processing data in fish eggs and larvae in the California Current Region. *U. S. N.O.A.A. Technical Report N.M.F.S. Circ.* **370:** 38 pp.

Kutkuhn, J. H. 1966. The role of estuaries in the development and perpetuation of commercial shrimp resources. *Spec. Publ. Am. Fish. Soc.* **3:** 16–36.

Kutkuhn, J. H., H. L. Cook, and K. N. Baxter. 1969. Distribution and density of prejuvenile *Penaeus* shrimp in Galveston entrance and the nearby Gulf of Mexico (Texas). *F.A.O. Fish. Rep.* **57:** 1075–1099.

Lachenbruch, P. A. 1975. *Discriminant analysis.* Hafner Press, Macmillan Pub. Co., New York. 128 pp.

Lysek, H., S. Komenda, N. Cuervo Pineda, and N. Lorenze Hernandez. 1975. Discriminant analysis in biometrical study of eggs of *Trichuris trichiura* and *Trichuris suis. Acta Univ. Palacki. Olomuc. Fac. Med.* **73:** 121–136.

McEwen, G. F., M. W. Johnson, and T. R. Folsom. 1954. A statistical analysis of the Folsom sample splitter based on test observation. *Archiv Meteorol., Geophys. Bioklimatol., Ser. A Metereol. Geophys.* **6:** 502–527.

Motoh, H. 1979. Larvae of decapod Crustacea of the Phillipines. III. Larval development of the giant tiger prawn, *Penaeus monodon*, reared in the laboratory. *Bull. Jpn. Soc. Sci. Fish.* **45:** 1201–1216.

Motoh, H., and P. Buri. 1979. Larvae of decapod Crustacea of the Phillipines. IV. Larval development of the banana prawn, *Penaeus merguensis*, reared in the laboratory. *Bull. Jpn. Soc. Sci. Fish.* **45:** 1217–1235.

Munro, J. L., A. C. Jones, and D. Dimitriou. 1968. Abundance and distribution of the larvae of pink shrimp (*Penaeus duorarum*) on the Tortugas shelf of Florida, August 1962–October 1964. *U. S. Fish Wildl. Serv. Fish. Bull.* **67:** 165–181.

Neal, R. A. 1975. The Gulf of Mexico research and fishery on penaeid prawns. Pp. 2–8 in *First Australian National Prawn Seminar, Maroochydore, Queensland, 22–27 November 1973.* P. C. Young, ed. Australian Government Publishing Service, Canberra.

Nie, N. H., C. H. Hall, J. G. Jenkins, K. Steinbrenner, and D. H. Bent. 1975. *S.P.S.S.: Statistical Package for the Social Sciences.* 2nd ed. McGraw-Hill Book Co., New York. 675 pp.

Pearson, R. E. 1964. Use of a discriminant function to classify North American and Asian pink salmon, *Oncorhynchus gorbuscha* (Walbaum), collected in 1959. *Int. North Pac. Fish. Comm. Bull.* **14:** 67–90.

Perkins, E. J. 1956. Preparation of copepods mounts for taxonomic work and for permanent mounts. *Nature* **178:** 1075–1076.

Racek, A. A. 1959. Prawn investigations in eastern Australia. *New South Wales State Fisheries Research Bulletin* No. 6. 57 pp.

Raje, P. C., and M. R. Ranade. 1972. Larval developments of Indian penaeid shrimp. I. *Penaeus merguiensis* de Man. *J. Indian Fish. Assoc.* **2:** 1–16.

Roessler, M. A., A. C. Jones, and J. L. Munro. 1969. Larval and postlarval pink shrimp *Penaeus duorarum* in south Florida. *F.A.O. Fish. Rep.* **57:** 859–866.

ROTHLISBERG, P. C. (1982). Vertical migration and its effects on dispersal of penaeid shrimp larvae in the Gulf of Carpentaria, Australia. *U. S. Fish Wildl. Serv. Fish. Bull.* **80**: 541–554.

ROTHLISBERG, P. C., AND C. J. JACKSON. 1982. Temporal and spatial variation of plankton abundance in the Gulf of Carpentaria, Australia 1975–1977. *J. Plankton Res.* **4**: 19–40.

ROTHLISBERG, P. C., J. A. CHURCH, AND A. M. G. FORBES (in press). Modelling the advection of vertically migrating shrimp larvae. *J. Mar. Res.*

ROTHSCHILD, B. J., AND J. A. GULLAND. 1982. Interim report of the workshop on the scientific basis for the management of penaeid shrimp. Key West, Florida November 1981. *N.O.A.A. Tech. Memorandum N.M.F.S.-S.E.F.C.-98.* 66 pp.

SAILA, S. B., AND J. M. FLOWERS. 1969. Geographic morphometric variation in the American lobster. *Syst. Zool.* **18**: 330–338.

SANDIFER, P. A., AND P. J. ELDRIDGE. 1976. A study of the distribution and abundance of *Penaeus* larvae off the southeastern United States in an attempt to identify spawning grounds of the brown shrimp. *Final Report State-Federal shrimp management program* Contract No. 03-5-042-16. Marine Resources Research Institute. South Carolina Wildlife and Marine Resources Department. 21 pp.

STAPLES, D. J. 1979. Seasonal migration of postlarval and juvenile banana prawns, *Penaeus merguiensis* de Man, in the major rivers of the Gulf of Carpentaria, Australia. *Aust. J. Mar. Freshw. Res.* **30**: 143–157.

SUBRAHMANYAM, C. B. 1971. The relative abundance and distribution of penaeid shrimp larvae off the Mississippi coast. *Gulf Res. Rep.* **3**: 292–345.

TEMPLE, R. F., AND C. C. FISCHER. 1967. Seasonal distribution and relative abundance of planktonic-stage shrimp (*Penaeus* spp) in the northwestern Gulf of Mexico. *U. S. Fish Wildl. Serv. Fish. Bull.* **66**: 323–334.

WICKINS, J. F. 1976. Prawn biology and culture. *Oceanogr. Mar. Biol. Ann. Rev.* **14**: 435–507.